Design, synthesis and antiproliferative activity of urocanic-chalcone hybrid derivatives

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Inspired by biologically active natural products, a hybrid analogue that combines the N-Me urocanic side chain of the sarcodictyin family of compounds with the chalcone motif has been proposed, synthesised and examined for antiproliferative activity in three cancer cell lines and one normal primary cell line. The analogues are all synthesised in one or two steps from commercially available materials and, of the compounds examined, the proposed hybrid analogue displays the most active and selective inhibition of cell proliferation in human colon cancer cell line HT29 (IC₅₀ 2.9 µM) and highly metastatic human breast carcinoma MDA-MB-231 (IC₅₀ 4.8 µM).

Introduction

Eleutherobin 1 and the structurally related sarcodictyin family of compounds A and B are natural products originally isolated from soft coral. They possess potent anti-tumour activity with a mechanism of action similar to paclitaxel (Taxol®) through the stabilisation of microtubules. ¹⁻⁸ They have attracted great interest as they display nanomolar cytotoxicity against a range of cancer cell lines including Taxol®-resistant cell lines.⁵⁻¹⁰ Their limited availability from corals has limited their use in the synthesis of analogues. ¹ⁱ⁻¹⁴

Fig. 1 Eleutherobin 1 and sarcodictyin A (2a) and B (2b)

Owing to their limited availability from corals, chemical synthesis is required to explore potential clinical application. The total syntheses of eleutherobin ¹¹⁻¹⁴ and sarcodictyins A (2a) and B (2b) ¹⁵⁻¹⁷ have been reported. Extensive structure-activity relationships of the sarcodictyins were conducted and found that the α,β unsaturated urocanic ester side chain was vital for biological activity. ⁸⁻¹⁷⁻¹⁸ Removal of this pharmacophore resulted in complete loss of cytotoxicity and even subtle substitutions of the imidazole ring were not tolerated and resulted in decreased activity.

Gennari has reported the synthesis of a variety of simplified eleutherobin analogues, using enantioselective allyltitanation reactions and ring closing metathesis (RCM) as key steps. ¹⁹⁻²³ The simplified analogues, exemplified by compounds 3 and 4, retained potent microtubule-stabilising properties (ED₉₀ effective dose that induces 90% tubulin polymerisation) but exhibited reduced inhibition of tumour cell proliferation (IC₅₀ concentration inhibiting cell proliferation by 50%) (Fig. 2). It was suggested that this may be the result of hydrolytic cleavage of the N-methyurocanic ester due to a lack of substitution at C7, resulting in diminished activities, as the natural products are fully substituted at the equivalent position. ²³ Although most studies show that the ester is essential to biological activity, ⁸⁻¹⁷⁻¹⁸ Holmes reported simplified eleutherobin analogues 5 and 6 which displayed improved tubulin binding activities when the N-methyurocanic ester side chain was absent. ²⁶

Fig. 2 Gennari²¹⁻²³ and Holmes²⁶ simplified analogues.

Since the urocanic ester is required for potency in the natural products and can be susceptible to hydrolysis in simplified
analogaes, we considered using ketone isosteres. Substituting the tricyclic core of the sarcodictyins with an aromatic group would generate compounds closely related to the structure of chalcones 7 that are biologically active compounds themselves.\textsuperscript{27} We therefore proposed to investigate chalcone derivatives that feature the \(N\)-methylocyclohexan moiety, such as hybrid 8 shown in Fig. 3.

Despite the wealth of research in the area of chalcones and related compounds, very few examples have been reported which possess the framework of the proposed hybrid compound 8. These include compound 8a \((R = H)\) generated as an intermediate in the synthesis of \(\gamma\)-lactam alkaloids.\textsuperscript{28} The structurally similar compound 9 in which the enone is conformationally constrained has been previously reported and exhibits potent and selective inhibition of \(P_{450}\) aromatase \((P450\ arn,\ CYP19)\), with possible therapeutic applications in various hormone-dependent cancers, such as breast, prostate and uterine (Fig. 4).\textsuperscript{29-33} Similar compounds have also been investigated as agonists of the \(\alpha_{2B}\) and \(\alpha_{2C}\) adrenergic receptors for the treatment of chronic pain.\textsuperscript{34,35}

The chalcone motif is a privileged structure present in an extensive range of biologically active molecules that display anti-inflammatory, anti-infective, anti-oxidative, anti-malarial and anti-cancer activities.\textsuperscript{27,36} Recent reports have shown chalcones having a trimethoxyphenyl group exhibit potent biological activities against various cancer cell lines, selected examples of which are shown by compounds 10,\textsuperscript{37} 11\textsuperscript{38} and 12\textsuperscript{39} in Fig. 4. The trimethoxyphenyl group is also present in a range of biologically active compounds such as colchicine 13,\textsuperscript{40} combretastatin A4\textsuperscript{41} and podophyllotoxin 15,\textsuperscript{42,43} all of which also bind to tubulin but induce depolymerisation (Fig. 4).

Colchicine 13 and podophyllotoxin 15 display limited clinical potential due to severe toxicity; however, the phosphate prodrug of combretastatin \((CA4P)\) is a promising lead compound under clinical evaluation.\textsuperscript{40,41,44} The success of combretastatin 14 demonstrates that structural complexity is not necessary for potent biological activity and that simple molecular scaffolds can provide new lead compounds. One emerging strategy in the development of new drugs involves combining key pharmacophores from existing natural products to generate novel chimeric compounds with improved biological properties.\textsuperscript{45-49}

Combining the pharmacophores of the trimethoxyaryl motif and the urocanic ester side chain, the synthesis and antiproliferative activity of various urocanic-chalcone hybrid derivatives is now described.

Results and discussion

Chemical Synthesis

The chalcone hybrid derivatives were obtained by a Claisen-Schmidt condensation between the methoxy-substituted acetophenones A, B and/or C and the heteroarylcarboxaldehyde 1,2,3,4 and 6-CHO (Scheme 1). N-Me imidazole derivatives C5 and C7 were obtained by methylation of the corresponding \(N\)-H imidazole, compounds C3 and C6 respectively.

Scheme 1 Synthesis of proposed urocanic-chalcone hybrid derivatives.
Table 1  Synthesis and inhibition of cell proliferation of various hybrid analogues

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R^1</th>
<th>R^2</th>
<th>R^3</th>
<th>R^4</th>
<th>Method^a</th>
<th>Yield (%)</th>
<th>IC_50 (µM)^b</th>
<th>HT29</th>
<th>MDA-MB-231</th>
<th>LNCaP</th>
<th>FEK-4</th>
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<td>H</td>
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<td>H</td>
<td>H</td>
<td>i</td>
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<td>&gt; 500</td>
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<td>OMe</td>
<td>H</td>
<td>H</td>
<td>i</td>
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<td>OMe</td>
<td>H</td>
<td>i</td>
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<td>H</td>
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<td>H</td>
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<td>OMe</td>
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<td>0.120^f</td>
<td>0.154^f</td>
<td>n.d</td>
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^a Methods (i) 1 equiv. LiOH.H_2O, (ii) NaOH, grinding, (iii) 2 equiv. BF_3.OEt_2, (iv) H_2, Pd/C, (v) NaH, MeI, DMF and (vi) Cs_2CO_3, MeI. ^b IC_50 is the concentration that inhibits 50% cell proliferation. Values are the mean from three independent experiments, except ^c two and ^d one experiment, with quadruplicate readings in each. ^e Reference 21, ^f paclitaxel IC_50<5nM. ^g Doxorubicin was used as a positive control, n.d not determined.

LiOH.H_2O has been reported to catalyse the Claisen-Schmidt reaction of various aryl methyl ketones with heteroaryl aldehydes under mild conditions. The reactions of 2-pyrolecarboxaldehyde 1-CHO with acetonophenones A, B and C using 10 mol% of LiOH.H_2O, as described, were unsuccessful, although using 1 equivalent gave the products A1, B1 and C1 in moderate yields (Method (i), entries 1-3, Table 1). Following recently reported solvent-free conditions, grinding 1-methyl-2-pyrolecarboxaldehyde 2-CHO with acetonophenones A, B and C in the presence of NaOH with a mortar and pestle gave the corresponding products A2, B2 and C2 after only 5 min. in moderate to good yields (Method (ii), entries 4-6, Table 1). Unfortunately, attempts to apply this method to other substrates failed.

The imidazole derivatives gave variable results under these conditions and treatment with 50 mol% of BF_3.OEt_2, as described by Narender, only gave starting materials, presumably because of unproductive co-ordination to the basic nitrogen in imidazole. Use of two equivalents of BF_3.OEt_2, followed by decomplexation with NaOH, gave the desired urocanic-chalcone derivatives A3, B3 and C3 in moderate to good yields (Method (iii), entries 7-9, Table 1).

To examine the effect of the enone double bond, the dihydro-derivative C3-H_2 was obtained in 57% yield by hydrogenation of the conjugated double bond of compound C3 under standard conditions (entry 10, Table 1). Having obtained the core framework of the urocanic-chalcone hybrid with acetonophenones A, B and C, further derivatives were focused on the trimethoxyaryl motif C. 1-Methylimidazole-2-carboxaldehyde 4-CHO is commercially available and gave the non-natural urocanic-chalcone 1-Me derivative C4 in the presence of BF_3.OEt_2 in 54% yield (entry 11). Methylation of the imidazole group present in C3 using Cs_2CO_3 and MeI gave a 50:50 ratio of 1-Me C4 and 3-Me C5 isomers, which proved difficult to separate. Using the conditions reported for the selective methylation of the N-urocanic methyl ester, NaH and MeI in DMF at 0 °C selectively gave the desired 3-Me isomer C5 in a 75:25 ratio of C5:C4 that could be separated by silica gel column chromatography (entry 12). The 2-substituted imidazole derivative C6 was obtained using excess BF_3.OEt_2 and was methylated under standard conditions to afford compound C7 without the formation of isomers due to the symmetry of the imidazole substituent (entries 13 and 14).
Cell Proliferation Assays

In all cases, the only stereoisomer observed was the thermodynamic trans-product, identified by a characteristic 1J coupling of ca. 15 Hz. All compounds were determined to be ≥ 95% pure by HPLC and were examined for inhibition of cell proliferation in vitro using the MTS assay with various cell lines and the results are reported as the concentration required to inhibit 50% cell proliferation (IC50) (Table 1). The results are the mean of three experiments, except where indicated in Table 1, and were performed on different days with different batches of cells. Three cancer cell lines were investigated, human colon carcinoma HT29, highly metastatic human breast carcinoma MDA-MB-231, and an androgen-dependent human prostate carcinoma LNCaP. In addition, a normal primary cell line, human skin fibroblasts FEK-4, were also investigated in the MTS assay to examine selectivity.

The addition of methoxy substituents significantly increased activity of the N-H pyrrole derivatives (A1-C1) and was also observed to a lesser extent with N-Me pyrrole hybrids (A2-C2) (entries 1-3 and 4-6, Table 1). The effect of the substituents was not evident with N-H 4-imidazole derivatives (A3-C3), which showed moderate levels of activity against all three cancer cell lines, but without much selectivity (entries 7-9). The importance of the enone was demonstrated by the dramatic loss of activity of the saturated derivative C3-H3 (entry 10). The non-natural methylated analogue C4 gave similar levels of activity to the unsubstituted compound C3, although the proposed N-methylurocanic trimethoxy chalcone hybrid C5, which has the side chain present in the sarcodictyins, did exhibit enhanced antiproliferative activity and selectivity, especially with HT29 and MDA-MB-231 (entries 11 and 12). Interestingly, the symmetrical N-H 2-imidazole hybrid C6 and the methylated derivative C7 showed similar levels of potency as hybrid C5 against HT29 and MDA-MB-231 and displayed improved activity against LNCaP, although they are much less selective and also inhibited cell proliferation in the normal primary human skin fibroblasts FEK-4 (entries 13 and 14).

Conclusions

Combining pharmacophores present in the sarcodictyin and chalcone family of compounds, the hybrid analogue 8 was proposed and synthesised together with related isomers. It is of interest that both sarcodictyin and chalcones interact with tubulin, although they have opposing effects on microtubule assembly. From our investigations however, the lead compound is indeed hybrid chalcone C5 which includes the N-methylurocanic side chain present in the sarcodictyins. It is the most active and most selective compound in the series investigated, displaying selective antiproliferative activity against HT29 and MDA-MB-231 cell lines.

It is worthy of note that the urocanic-chalcone derivative C5 can be obtained in only two synthetic steps and displays similar levels of antiproliferative activity against HT29 as the simplified eluetherobin analogue 3 previously reported (Fig. 2 and Table 1, entry 15). Owing to their ease of synthesis, low molecular weight and promising initial results, these hybrid compounds could be used in a fragment-based lead discovery (FBLD) approach to achieve more potent compounds.

Hybrid C5, together with others examined in this series, will be further investigated to determine the biological mode of action and will be reported in due course.

The simple chalcone scaffold combined with the N-methylurocanic ester side chain present in the sarcodictyin family of compounds has afforded biologically active hybrid analogues with the potential for improved biological activities and drug-like properties due to their ease of synthesis and the diverse range of inexpensive and commercially available starting materials.

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Notes and references
